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1917

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T. W. GALLOWAY,
Secretary-Editor.

Beloit, Wisconsin.

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TRANSACTIONS
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American Microscopical Society
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Vol. XXXVI JULY, 1917 No. 3

THE ENCHYTRÆIDÆ (OLIGOCHÆTA) OF THE WOODS HOLE REGION, MASS.*

By Paul S. Welch

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Introduction

During the summers of 1915-16, the writer carried on some experimental studies with certain Enchytræidæ (Oligochæta) at

*Contribution from the Entomological Laboratory, Kansas State Agricultural College, No. 29.

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Woods Hole, Mass. Owing to the fact that the known marine species of that locality intermingle in the same habitats, and that some of them have been reported from both fresh-water and terrestial conditions, it was necessary to identify the enchytræids from the various collecting grounds about Woods Hole in order to avoid error in handling immature individuals and to insure accuracy in the identity of the materials used. In spite of the fact that two species occur in great abundance in the environs of Woods Hole and certain other parts of the Atlantic Coast, they have received scant attention from workers in any of the fields of zoology.

The writer wishes to acknowledge indebtedness to Director F. R. Lillie for the privilege of using the facilities of the Marine Biological Laboratory during the time this and other related work was in progress.

Enchytræus albidus Henle

Previous Records

Four records of this species on the Atlantic Coast occur in the literature. Verrill ('73) was the first to discover it in North America, describing it as *Halodrilus littoralis,* but its identity with *Enchytræus albidus* Henle was later established by Michaelsen ('00, p. 89). Verrill reported it from "New Haven; Woods Hole; Casco Bay, Maine; very common under dead sea-weeds and stones near high-water mark." Smith ('95) made a careful anatomical study of this annelid at Woods Hole, using the name *Enchytræus littoralis* Verrill. He found the specimens extremely abundant in the same kinds of situations described by Verrill. Moore ('05) reported *Enchytræus albidus* very numerous at Woods Hole as well as along the coast from Casco Bay, Maine, to Sea Isle City, New Jersey. He not only found them in the inter-tides zone of the shore but also "in moist spots on farm lands on Martha's Vineyard, where it could readily be introduced in the large quantities of eel grass that are annually spread for fertilizer." He likewise states that "about Woods Hole it also lives in damp, sandy woods and on the shores of fresh-water ponds, especially of one that formerly was connected with the Sound." Passing notice is made of all of the above-mentioned records by Sumner, Os-

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burn, and Cole ('13). Hunt ('15) collected this species "in abundance from the coarse gravel of the tidal zone on the seashore at Cold Spring Harbor, Long Island, New York."

Habitat

Specimens of *E. albidus* were found in great quantities in several of the protected coves about Woods Hole where the tide casts up masses of eel-grass on sandy or gravelly beaches. They appear to be restricted entirely to the inter-tides zone since no specimens were collected in the comparatively dry sand above the high tide line and none was observed beyond the low tide limit where the sand is constantly covered with water. At low tide, the windrows of eel-grass retain a considerable amount of moisture, thus keeping the underlying sand saturated and protecting it from the heat, light, and drying effect of the summer sun. At high tide, the entire zone occupied by these worms is submerged. They were absent from sandy beaches which lacked the masses of eel-grass, and even when the latter were present, the worms were apparently much reduced in numbers when the sand contained little or no organic debris. In July and August, these enchytræids occur in great numbers on the beach just opposite the Lecture Hall of the Marine Biological Laboratory. The principal features of this habitat are a smooth, sandy-gravelly, gently sloping beach; thick layers of eel-grass on the inter-tides zone at low tide; quantities of fine pieces of coal and cinders mixed with the sand; large amounts of organic matter in various stages of decomposition; and complete protection from wave action. The worms have a rather definite vertical distribution. In the matted eel-grass, they usually occur in very small numbers, but appear in large quantities in the first one to three inches of underlying sand and gravel. Below these depths, the number decreases very rapidly. They show a distinct tendency to accumulate about small rocks and pebbles which are imbedded in the sand so that when such an object is removed, the walls of the cavity are frequently covered with worms.

Several examinations of the various brackish, fresh-water, and moist woodland habitats did not reveal the presence of these enchytræids, although Moore ('05, pp. 394-395) reported them in such places. However, it is very possible that a more thorough search

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would reveal them under the above-mentioned conditions, since in addition to Moore's record they are definitely known from such habitats in other parts of the world.

Methods of Culture

Specimens were easily kept alive in the laboratory by placing a number of them in shallow stender dishes containing sea water to the depth of about one centimeter, to which had been added pieces of fresh, green eel-grass from the drift on shore. In most of the cultures used by the writer, the eel-grass was partly cleaned by washing and wiping each blade, in order to avoid introducing into the culture an excess amount of the extraneous matter adhering to the eel-grass which interfered with the examination of the cultures for cocoons. All of the cultures were kept covered, some being kept in darkness while others stood in diffuse sunlight. In such cultures, the specimens were active, cocoons were produced, and the mortality was very low, the worms living until the cultures were purposely destroyed, in some cases, after thirty days. Should it be desirable, the water in the cultures can be reduced to the amount necessary only to keep the worms and eel-grass thoroughly moist. Hunt ('15, pp. 496, 501, 503) cultured this same enchytræid for regeneration experiments by placing them "in small sterilized glass bottles, each containing a strip of filter paper and enough sterilized sea water to keep the animals well moistened." He also found that the worms could be kept in such bottles moistened with "fresh water" in which they regenerated as well as in the salt-water environment. It would appear from Hunt's statement of methods that his specimens were not *submerged* and he makes no statement as to the source of the "fresh water." The writer was unable to rear these enchytræids at Woods Hole in the fresh water taken from the laboratory supply. In no case did the worms, when submerged, live more than eighteen hours. However, the addition of a very small amount of sea water to the fresh water made the culture a fairly favorable one.

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LUMBRICILLUS LINEATUS (Müll.)

Identity

The final decision as to the identity of this annelid was reached after the completion of a detailed study of the anatomy and a thoroughgoing examination of the literature dealing with a number of European species which seem to exhibit various degrees of mutual affinity. The complex character of the identification was due to the existence of a number of very similar descriptions of forms under several different names. The writer believes that this study of Woods Hole material has helped to solve some of the difficulties and to establish the identity of certain so-called species. While access to the European material has not been possible, the descriptions, discussions, and figures of the morphology of these foreign forms are sufficiently detailed to serve as a working basis for comparison.

The anatomy of these worms agrees very closely with the description by Moore ('05, pp. 395-397) of a form which he collected at Woods Hole and named *Lumbricillus agilis*. The only points of disagreement have to do with the position of the clitellum, the position and number of the ventral glands, and the character of the spermathecal glands. These matters will be discussed later under their respective headings and the apparent discrepancy accounted for, at least in large part. It will suffice to state here that there seem to be no grounds for a possible suggestion that the material studied by Moore represented a species different from the one examined by the writer.

An inspection of the descriptions of other species ascribed to *Lumbricillus* reveals at least four foreign ones which, as described, approach the Woods Hole form too closely to be disregarded, namely, *litoreus* (Hesse), *lineatus* (Müll.), *subterraneus* (Vejd.), and *verrucosus* (Clap.). That some of the above-mentioned species are not valid has been suspected before. Southern ('09, p. 150) pointed out that the differences between *litoreus* and *lineatus* seemed of no great importance and that it "is doubtful whether there is sufficient justification for keeping the two species apart" and Michaelsen ('01 a, p. 207) has indicated the probability that *lineatus* and *subterraneus* should not be separated. Friend ('16, pp. 23-24), in the examination

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of specimens from the sewage works of Manchester, England, found that "every intermediate stage" between *verrucosus* (Clap.) and *lineatus* could be detected. With respect to *verrucosus,* the only points of distinction of any importance between it and *lineatus* have been the number of setæ per bundle and character of the ventral glands. A careful parallel column comparison of the characters of all the above-mentioned enchytræids, together with those of the Woods Hole specimens, shows that the range of variability of the body length, setæ, origin of the dorsal blood-vessel, shape of the margins of the brain, dimensions of the spermiducal funnel, and number and development of the ventral glands are such as to link all of these so-called species together. A detailed comparison is out of place here, but reference to the earlier descriptions and a comparison with the following discussion of the morphology of the Woods Hole material will bear out this conclusion.

Since *L. litoreus* (Hesse), *L. subterraneus* (Vejd.), *L. lineatus* (Müll.), *L. verrucosus* (Clap.), and *L. agilis* Moore must all be regarded as the same species, the question arises as to what name shall persist to designate it. It appears that *lineatus* (Müll.) (1771) has priority, but Ditlevsen ('04, p. 431) holds that *lineatus* should give place to Levinsen's name *rivalis* (1884): "Michaelsen hat gewiss recht zu sagen, dass die Art von Levinsen dieselbe wie *Pachydrilus germanicus* Mich. ist; aber nichts in der Beschreibung von O. Fr. Müller berechtigt ihn dazu, diese mit *Lumbricus lineatus* O. Fr. Müller zu identifizieren. Die Charaktere, welche O. Fr. Müller angibt, passen nur gemeinsam auf die Gattung *Pachydrilus*; es ist aber durchaus unmöglich einige ihrer Arten danach zu bestimmen. Da Levinsens Beschreibung die älteste ist, muss die Art den Namen *Pachydrilus rivalis* behalten, und die Art Michaelsens muss dieser zugeordnet werden." The writer has examined the description by O. F. Müller and its vagueness and lack of specific detail is certainly apparent, giving ample reason for the numerous attempts to place this uncertain *"Lumbricus lineatus."* The description might apply to almost any of the species assigned to *Lumbricillus.* On such grounds it might appear that Ditlevsen's contention has some basis but it is evident that, if the synonymy proposed in this paper is correct, the name could not be *rivalis* Levinsen since the

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name *verrucosus* has priority over it. At the present time there seem to be grounds for regarding *verrucosus* as the valid name, but since some uncertainty still exists concerning some of the other apparently closely related species, the writer prefers to retain, for the present, the old name of *lineatus*.

There is growing evidence that an overindulgence in "species making" has occurred in the genus *Lumbricillus* and the writer is convinced that at least two other so-called species, and probably more, are the same as *lineatus*. The genus needs extensive revision and, when worked over in connection with complete and dependable morphological data, it is very probable that the number of species which the genus is now supposed to contain will be materially reduced.

With respect to the geographical distribution, these annelids have been reported under the old names as follows: *L. lineatus*: Denmark, Germany, North Russia, and England. *L. litoreus*: Italy and Ireland. *L. verrucosus*: Hebrides, Terra del Fuego, Switzerland, Ireland, Scotland, and England. *L. subterraneus*: England, Ireland, Scotland, France, and Bohemia. *L. agilis*: Casco Bay to Vineyard Sound, Mass. Evidently this species is a cosmopolitan form of wide distribution.

Habitat

In connection with the above rearrangement, it is interesting to consider, under the old names, the habitats from which these animals have been reported. *L. lineatus* has been recorded from debris and under stones on the seashore, or river margins, and on aquatic plants in fresh-water ditches. *L. litoreus* was reported on the seashore under stones. *L. verrucosus* has been found on the seashore under stones, in fresh-water situations, and, with some uncertainty, from earth near manure heaps. *L. subterraneus* has been collected in springs and aqueducts, in sewage works, in streams contaminated with wastes, and on the seashore near high-tide mark. It thus appears that all of the hitherto supposedly different forms occur on the seashore under similar conditions, and in addition certain fresh-water habitats may be occupied, indicating a considerable range of toleration for differences in the presence or absence of salt. In fact,

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Stephenson ('11, p. 39) found that certain seashore forms from Fintry Bay, which he recorded under the name *L. subterraneus,* "live well for several days in a mixture of equal parts of salt and fresh water, and equally well in altogether fresh water."

Specimens of *Lumbricillus lineatus* were abundant, during July and August, in a number of situations about Woods Hole, frequently intermingling with specimens of *Enchytræus albidus.* In general, the conditions of the habitats of the two species are much the same. Protected coves with sandy, gravelly or rocky, sloping beaches, on which the windrows of eel-grass accumulate near the upper tide limit, provide favorable grounds for these worms. They seem to prefer moderately moist conditions since the larger numbers occur near high-water mark, and seldom at or below the low-tide limit. Their usual position is just below the surface of the moist sand or gravel and there is a distinct tendency to accumulate about the imbedded stones or other larger objects to which they cling closely. Occasionally, just after high tide, specimens may be found on the surface of the sand below the overlying eel-grass which affords a protection against sunlight and retains ample moisture to bridge over the low-tide periods. However, specimens were found on semi-rocky beaches where the eel-grass was quite thin or almost absent but the worms were invariably deeper in the sand. The writer found this worm very abundant in the protected coves in the vicinity of Hadley Harbor. They also occurred in considerable numbers, intermingling with *E. albidus,* on the beach just opposite the Lecture Hall of the Marine Biological Laboratory. The two species can usually be distinguished in the mature stage by the smaller size and particularly by the reddish color of *L. lineatus, E. albidus* being invariably milk white. However, it requires more careful scrutiny to distinguish with certainty the immature forms since those of *L. lineatus* are practically colorless.

Methods of Culture

These worms are readily kept alive in the laboratory for indefinite periods by employing the methods already described for *E. albidus.*

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Morphology

Studies of the external and internal anatomy of these worms corroborate, in most respects, the work of Moore. However, there are some points of disagreement and, in addition, certain structures and organs are described in detail for the first time. Therefore, only those features of the morphology which demand special discussion will receive consideration here and it will be understood that otherwise the findings of the writer are in agreement with those of Moore.

Clitellum.—A well-defined clitellum is present in sexually mature specimens. Moore states that it "extends completely around somites XI and XII." This statement is evidently an error since none of the numerous specimens examined by the writer has the clitellum in that position but instead it occurs on XII and XIII. Its completeness on the ventral side demands some additional description. Transverse sections through the penial bulbs or in their immediate vicinity show the clitellum incomplete on the ventral side but sections cephalad or caudad of the penial bulbs demonstrate that this incompleteness is only partial and that the two ends of the clitellum are continuous, although distinctly thinner on the ventral surface.

Brain.—The description and figure given by Moore ('05, p. 396; fig. 24) present correctly the main features of this organ with the exception that only one pair of supporting strands are represented, those extending from the posterior lobes. In reality, another pair of slender supporting strands extends from the lateral margins, near their middle, diagonally laterad to make attachment with the body-wall. The posterior emargination, the angular form of the posterior lobes bounding the emargination, and the very shallow concave form of the anterior margin seem to be constant characters. The general dimensions of the organ as a whole are subject to some variation, the proportion of the length and width varying from 1.25:1 to 1.75:1.

Spermiducal Funnel.—The spermiducal funnel requires no additional description save in connection with the length. Moore ('05, p. 397) states that this organ is about six to eight times as long as thick. The writer has found considerable variation in the length, depending upon the state of extension or contraction of the animal.

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In fully extended specimens, the above-mentioned dimensions are approximately correct, but most of the specimens exhibit funnels the length of which is only about four times the diameter.

Penial Bulb.—The external termination of the sperm duct is described by Moore ('05, p. 397) as opening "into the small, depressed, spheriodal, glandular and opaque atrium, which itself opens on the medial side of a small bursa in the position of the ventral seta. The bursa can be everted as a conical penis." Properly translated into the more modern terminology, the writer finds this very brief description of the penial bulb apparatus to be correct as far as it goes. The term "atrium" is applied to the organ which the writer and others designate as the *penial bulb,* atrium being retained to designate the enlargement of the sperm duct which appears in certain genera of enchytræids just entad of the penial bulb. The "bursa" evidently refers to the penial invagination.

Structurally, the penial bulb is simple and conforms to the lumbricillid type. Its transverse diameter is about one-fifth that of the body in the same region. It is approximately globular and enclosed in a thin musculature. The interior is filled with glandular cells of one kind only. They differ somewhat in shape in the different parts of the bulb but in general they are elongate, closely set together, and most of them are arranged radially about the penial lumen. The cells are distinctly nucleated, the nuclei being located for the most part in the peripheral ends of the cells, although they appear in limited numbers nearer the penial lumen and well within the interior of the bulb. The cytoplasm of the bulb cells takes artificial stains with moderate intensity only. The sperm duct unites with the dorsal part of the bulb near the body-wall and the former merges so gradually into the latter that it is not possible to locate exact limits.

Ventral Glands.—These problematical organs, designated in the literature under various names, such as "Kopulationsdrüsen", "copulatory glands", "outgrowths of the ventral nerve cords", "ventral glands", occur in this species but are subject to considerable variation in mature specimens. Well-developed glands occur in XIII and XIV and sometimes in reduced form in XV. Most specimens contain the first two but the one in XV appears only occasionally. The shape and degree of development are also strikingly variable. Speci-

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mens were examined in which the glands in XIII and XIV are produced into long wings extending dorso-laterad on either side of the nerve cord, while the one in XV is diminutive and easily overlooked, not extending above the level of the dorsal surface of the nerve cord and developed mainly on one side only. Other specimens show only the gland in XIV well developed and the one in XIII much smaller, both being developed ventrad and laterad of the nerve cord but not into wings, while no gland appears in XV. In all cases, the glands are closely associated with the nerve cord, completely surrounding it except for the median dorsal surface which is exposed. Moore ('05, p. 396) states that the "copulatory supra-neural glands are well developed, especially in somites III, IV and V. They are slender and elongated, not closely united with the ventral nerve, and open on each side nearly at the ventral setæ bundles." The writer has found nothing in III, IV, and V which can be identified as ventral glands, the only thickenings of the nerve cord occurring in those somites being due to the ganglia themselves.

Ovisac.—In the mature specimens in which there is an accumulating mass of developing eggs, the septum XII/XIII is pushed caudad to the posterior part of XIII. Whether this can justly be designated as an ovisac may be open to question, but it probably represents the diminutive "ovisac" reported by Moore.

Spermathecæ.—The spermathecæ are large, well-developed organs lying in the usual position in V. Each is distinctly sigmoid in shape and opens independently into the dorso-lateral aspect of the digestive tract. There is no distinct demarcation between the duct and the ampulla. The latter bears no diverticula, is fusiform in shape, and contains a rather spacious lumen which corresponds to the shape of the exterior. Both ectal and ental openings are distinct and larger than usual. A complete crown of conspicuous glands surrounds the ectal end of the duct and opens into the lumen of the latter at or just entad of the external surface of the body. This crown surrounds practically the whole spermathecal duct, but is not directly united with it except as above mentioned. The ental border of this crown of glands is emarginate at more or less regular intervals, giving it a lobulate appearance and suggesting its origin from a number of glands developed in very close proximity. This crown is made up

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of a large number of cells and each lobule is also multicellular. It must be noted in this connection that Moore ('05, p. 397) found the ectal end of each spermatheca surrounded by a "circle of glands chiefly aggregated into an anterior and a posterior group." His figure of the same (Fig. 28) shows a very irregular crown of *unicellular* glands, but the writer is inclined to suspect that imperfectly mature specimens might have been studied.

Fridericia ratzeli (Eisen)

Identity

Worms, belonging to this species, were collected from the earth and accumulating humus of the forest floor in Fay's woods, back of the village of Woods Hole. Difficulties arose in connection with the identification of these enchytræids, the chief obstacle being the far too common one, namely, the vagueness and incompleteness of certain European descriptions. A complete morphological study shows that, with one minor exception, there is close agreement with those characters of *Fridericia ratzeli* (Eisen) which have been described, but, unfortunately, the anatomical discussions of the latter fail to give any data concerning the following important features: origin of the dorsal blood-vessel; position of the clitellum; extent and structure of the peptonephridia; the chylus cells; and the structure of the penial bulb. In spite of these deficiencies, there seems no other alternative, at present, than to consider the Woods Hole specimens as belonging to *ratzeli*. The only point of disagreement is the origin of the efferent duct of the nephridium near the posterior end of the postseptal region, instead of near the septum as is described for the foreign specimens of this species. The peptonephridia in the Woods Hole specimens have fewer branches than seem to be called for in the descriptions but it is very doubtful if any significance can be attached to this variation. In addition, the posterior margin of the brain is truncate rather than convex, but such variation is known to occur in other species of Enchytræidæ. It seems, therefore, that the variations presented by the Woods Hole specimens are only of minor importance and not of sufficient weight to demand a different identification. There is, of course, the possibility that when the foreign

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representatives of *F. ratzeli* receive the much needed intensive morphological study that a varietal difference between them and the specimens from Massachusetts may appear and, in order to facilitate future comparisons, the morphology of the latter will be given in some detail.

It should be noted that the specimens from Woods Hole correspond very closely to an American species, *F. californica,* described in detail by Eisen ('05, pp. 119-121) from specimens collected in the vicinity of San Francisco, although certain points of disagreement appear, such as the origin of the dorsal blood-vessel and the origin of the nephridial duct. The chylus cells and the penial bulb are evidently of the same structure in the California and Woods Hole specimens. The writer is forced to the conclusion that *californica* is identical with *ratzeli,* or if not identical, of no higher rank than that of a variety.

This description records the second appearance of *F. ratzeli* in North America, assuming that the writer is correct in placing *californica* Eisen as a synonym of this species. The two localities represented are separated by the width of the continent but this might be expected since *F. ratzeli* is widely distributed, occurring in England, Ireland, Scotland, Norway, Denmark, Germany, Switzerland, and Italy. The habits of this annelid appear to be much the same in the different parts of its geographical range, being found only in habitats of rich earth or damp moss.

External Morphology

The body is elongate, cylindrical, smooth, gradually decreasing in diameter caudad of the clitellum. In nine sexually mature worms, the length varies from 15 to 20 mm., the average being approximately 18 mm. The diameter varies from 0.42 to 0.59 mm. Except in the extreme anterior and posterior ends, the intersegmental grooves are indistinct. The number of somites varies from 55 to 64, the average being 62. A well-developed clitellum occupies XII-XIII. The setæ bundles have the usual characters of the genus *Fridericia.* Anterior to the clitellum, the ventral bundles contain 4-6 setæ, usually 6, while the lateral bundles have a similar number. Caudad of the

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clitellum, the bundles contain 2-5 setæ, the smaller groups occurring near the posterior extremity. A head pore is present on 0/I and dorsal pores begin on VII. The color of the living specimens was very light yellow to milk white.

Internal Morphology

Brain.—This organ occupies a median, dorsal position in I and II, chiefly in the latter. The anterior margin is distinctly convex, the posterior margin truncate, and the lateral margins convex, the maximum width being at a position about two-thirds the total length from the anterior end. The length is about one and one-half times the greatest width, typical measurements being as follows: length, 0.148 mm.; maximum width, 0.104 mm. Two pairs of supporting strands extend from the organ to the body-wall, one from the lateral margins near their middle, and the other from the caudo-lateral angles. The description of *F. ratzeli* based on European specimens calls for a convex posterior margin but since it is known that the brain is subject to change of form under different conditions of contraction and extension, this variation can be of little significance.

Dorsal Blood-vessel.—In the specimens examined, the exact origin of the dorsal blood-vessel is a little difficult to determine. It appears to become distinct from the perivisceral blood-sinus in XX, or in the adjoining somites. The descriptions of *ratzeli* fail to include any statements as to the origin of this vessel in the European specimens. Eisen ('05, p. 120) states that in *californica* it originates in XVI.

Peptonephridia.—A pair of these organs occurs ventrad of the digestive tract, one on the right side and one on the left. Each opens separately into the alimentary canal in the extreme anterior part of V and is confined to that somite. Sparse branching occurs at the base and at the extremity of each. Aside from the branching, the body of each gland is irregularly tuberculate. It is a matter of some difficulty to determine just how closely these organs correspond to the descriptions of foreign specimens of *ratzeli*. Michaelsen ('00, p. 100) states that they are "mehrfach verzweigt". Southern ('09, p. 164) finds them "freely branched" in specimens of *ratzeli* var. *bed-*

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dardi from Ireland. Eisen ('05, p. 120) describes them in *californica* as "narrow, slightly and irregularly branched", although his figure (Fig. 81, e) shows more branching than might be inferred from his statement. The peptonephridia are known to be subject to a certain variation within a species and the relatively slight branching in the Woods Hole specimens may merely represent one of the extremes.

Chylus Cells.—The chylus cell region of the intestine occupies XIV-XVI. Cells of two kinds are present, the chylus cells and the ental epithelial cells. The former are somewhat flask-shaped, the broader ends being ectad. The base of each is approximately truncate and the sides converge gradually entad. The ental part of the intracellular canal is usually straight or nearly so, but the basal portion varies somewhat in its course, being usually bent to one side of the cell and then curving abruptly, forming a right angle. The basal part of the canal thus lies more or less parallel to the base of the cell of which it is a part. This intracellular canal is lined by a relatively thick specialized layer of cytoplasm which is everywhere of uniform thickness and structure. The canal is ciliated for a part of its length. A well-developed blood-sinus is present, surrounding the base of each chylus cell and extending up the sides for about two-thirds of its length. A conspicuous nucleus lies in the base of each chylus cell, often a little to one side and in the angle formed by the chief bend of the intracellular canal. The ental epithelial cells lie between the apices of the chylus cells, the ental surfaces bounding a part of the lumen of the intestine and bearing a thickly set coating of elongate cilia. Interstitial cells are absent. The chylus cells are somewhat longer in XIV than in XVI, making the whole layer thicker in the anterior part of the chylus region.

A certain resemblance exists between the structure of the chylus cell region in the Woods Hole specimens and in that described for *californica* by Eisen. The exact position of the chylus cells, the character of the intracellular duct, the general shape and character of the ental epithelial cells, and the relative thickness of the layer are the same, but lack of agreement exists in the California specimens in the restriction of the blood-sinus to the bases of the cells, the close apposition of the cells, and the approximately parallel sides. Chylus

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cells do not seem to have been described for the European specimens and comparison is not possible.

Nephridia.—The anteseptal and postseptal parts are about the same size although some slight variation occurs in the different somites. The efferent duct arises from the mid-ventral surface of the postseptal part, near the posterior end. Some variation also exists as to the origin of this duct but it was not found farther forward than the middle of the postseptal region. The internal structure is very similar to the usual type found in the genus *Fridericia.*

Foreign specimens of *ratzeli* are described as having the origin of the nephridial duct on the anterior portion of the postseptal part near the septum. Eisen's description of the nephridia in *californica* contains no statement as to the exact origin of the duct but his figure (Fig. 81, a) shows it as arising near the middle of the postseptal region.

Spermiducal Funnel.—A pair of these organs lies in the usual position in XI with the bases close to the ventral part of X/XI and the free extremities directed cephalad. The length of each is about two to three times the maximum diameter. A small, reflected collar is distinctly set off from the body of the funnel by a constriction, its diameter being only about one-half that of the body of the funnel. Each sperm duct is confined to XII and presents a mass of convolutions in the ventral part of the cœlom.

Penial Bulb.—Structurally, the penial bulb is of the lumbricillid type. It is small, compact, globular, and completely invested by a thin but definite musculature which is derived from the muscle-layer of the body-wall. When completely retracted, the invagination is longer than the transverse dimension of the organ, the latter lying completely on the mesal side. The sperm duct unites with the penial bulb on the dorso-ectal surface, penetrating it for a short distance. Within the bulb, it is replaced by the penial lumen, which, curving abruptly laterad, extends to the penial invagination, opening into its ental part. The bulb is composed of cells of but one kind, these being large compared with the size of the organ and distinctly nucleated.

The structure of the penial bulb is similar to that described by Eisen ('05, p. 120) for *californica* in being composed of cells of only

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one kind, but in the retracted condition, the sperm duct cannot be said to "enter the bulb near the base, splitting the bulb into two unequal parts." Furthermore, if Eisen's figure (Pl. XV, Fig. 9) was made from a transverse section of the worm, a distinct difference appears in the shape of the invagination and the organ as a whole. Mention of the bulb ("prostate") in the descriptions of the European specimens is so brief that little can be done in the way of comparison. It is said to be almost as large as the spermiducal funnel, a proportion which in the Woods Hole specimens would make the bulb several times its present size.

Spermathecæ.—A pair of well-developed spermathecæ is present in V, each organ being composed of three regions: duct, diverticula, and ampulla. The ectal opening of the duct is in the usual position in the lateral aspect of IV/V and has associated with it two small, multicellular, pyriform glands. The ampulla bears on its ectal end a circle of irregular, sessile diverticula, usually eight in number, which vary somewhat in shape but are mostly globular and pendant. Excluding the expanded portion bearing the diverticula, the ampulla is distinctly spindle-shaped. Each ampulla opens independently into the dorso-lateral aspect of the digestive tract. In the sexually mature specimens examined, the ampullæ contained spermatozoa and, in some cases, spermatozoa were not only in the process of passing into the lumen of the alimentary canal, but masses of them were found well within it.

Ventral Glands.—In XIII, XIV, and XV, certain problematical glands occur in connection with the ventral nerve cord. They completely surround the ventral and lateral parts of the latter, leaving only the dorsal surface exposed. At first sight, they appear as unusually developed ganglia, but stained, transverse sections show the tissues of the nerve cord to be distinctly different from those which immediately surround it. These glands are made up of large, nucleated cells, massed irregularly together, which give off processes extending ventrad through the body-wall to the hypodermis. The latter assumes a finely striated appearance with hypodermal nuclei appearing only at irregular intervals. These gland cells are many times larger than the nerve cells immediately above them. The

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organs are not of uniform size, the one in XIII being larger than the subsequent ones.

Similar glands, described under the various names mentioned on a previous page, occur in certain other genera of enchytræids, namely *Lumbricillus* and *Marionina* (Welch, '14, pp. 150-151), but their appearance in *Fridericia* is quite unusual, being known only in two or three species. Ude ('01, pp. 6-7) found one ventral gland in XIII in the specimens of *ratzeli* which he examined. They have been reported in XIII-XV in *F. antarctica* Bretscher, a synonym, according to Michaelsen ('00, p. 100), of *ratzeli*. *Fridericia dura* (Eisen), another synonym of *ratzeli*, according to Michaelsen ('00, p. 100), but a distinct species according to Ude ('01, pp. 6-7) possesses one ventral gland in XIII.

It should be noted in passing that, in the Woods Hole specimens, superficially similar swellings of the ventral nerve cord occur in II, III, and IV. However, a study of sections through these anterior swellings shows that they are nothing more than ganglionic assemblages of nerve cells and not special masses of gland cells.

Summary

1. The enchytræid fauna of the Woods Hole region, as known at present, comprises three species, *Enchytræus albidus*, *Lumbricillus lineatus*, and *Fridericia ratzeli*. The first two are marine and occur in great abundance in the inter-tides zone, especially in the protected coves which accumulate windrows of eel-grass. The other species is a terrestrial form, inhabiting the soil and humus of forest land.

2. Both of the marine species can readily be cultured indefinitely in the laboratory by placing them in shallow stender dishes containing a small amount of sea water and pieces of green eel-grass from the drift on shore. They could not be kept alive in the fresh water taken from the Marine Biological Laboratory supply, although when a few drops of sea water were added the unfavorable conditions were relieved.

3. Detailed study of the morphology of the sexually mature *Lumbricillus* yielded evidence that the foreign forms which have been discussed under the names *lineatus*, *subterraneus*, *verrucosus*,

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and *litoreus* are all one species, and that the American species, *agilis*, originally reported from Woods Hole also has the same fate. These should all be regarded as one species under the name *lineatus* which has priority.

4. *Fridericia ratzeli* has evidently been discovered in North America before, under the name of *californica*, which was applied to specimens found near San Francisco by Eisen. Critical morphological study of the Woods Hole specimens makes it almost certain that *californica* is a synonym of *ratzeli*.

5. All of the three enchytræids herein mentioned are widely distributed in the Old World and evidently so in North America.

6. Morphological detail not readily summarizable are presented for two of the species. Special consideration has been given to the organs of the complex reproductive system, the chylus cells of the digestive tract, and the ventral glands.

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CHEMICAL MICROSCOPY*

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A speaker who has the temerity to address a joint meeting of two different technical societies always finds himself in an awkward predicament. He feels that he must present his subject from the viewpoint of each group of men, that he must lay equal emphasis upon all branches of the sciences represented by his audience. I find myself very much embarrassed, realizing this, and in doubt whether I am here in the guise of a microscopist or of a chemist.

This, gentlemen, is the introductory paragraph of the paper I had originally prepared. Since that time we have entered the great war and I know where I stand. I come to you as a chemist to make an appeal for a wider and more intelligent application of the microscope in every day chemical practice.

If the talk appears rambling and fragmentary I trust you will bear with me, for with several momentous issues on the hands of my department I have had little opportunity to prepare a new paper and none to make new lantern slides. I will, however, attempt to stick to my text—Chemical Microscopy. To my mind there is no such thing as microchemistry as opposed to macrochemistry and the term microchemical methods is a misnomer. A microchemical reaction or test may be one performed upon minute amounts of material without necessarily having recourse to the microscope.

Chemical Microscopy on the other hand requires that some type of magnifying optical instrument enters into the work. By chemical microscopy, therefore, we simply mean the application of microscopic methods to the solutions of problems arising in the chemical laboratory or in the chemical industries.

No instrument at our command can do so much or throw so much light upon obscure problems with so little expenditure of time, labor and material. We chemists have been wasting golden hours and slaving over sloppy methods to accomplish ends which could

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have been reached easily and leisurely and with a degree of certainty unsurpassed by anything we have had at our command with test tubes and beakers. Why spend hours upon a qualitative analysis that can be better done through the medium of the microscope in several minutes?

The time has come when we can no longer be satisfied with time-consuming operations. Industries must be speeded up, production increased, better inspection methods introduced, quality thereby raised and final cost in consequence reduced. I am simply preaching good conservation. If we fail in this war and disappear as a nation it will be because we have failed in our industries to produce the necessary material and the requisite quality. I cannot recall a single great industry today where microscopic methods intelligently applied will not lead to more or less marked improvements.

The causes of our failure fully to appreciate the value of microscopic methods are not hard to find. In the first place in the educational system of our chemists no adequate training has been given in the multiplicity of uses of the microscope and its potential industrial applications. In the second place too much emphasis has been laid upon biologic microscopy; so that the generally accepted view is, that this instrument is intended for studies in biology or medicine. As a result, the development of the modern so-called high grade microscope has followed strictly biological lines and has drifted farther and farther away from stands applicable for general work in the chemical laboratory. For example the best of our present day stands no longer have the mirror mounted upon a swinging bar capable of movements far to one side, or even above the stage for oblique illumination. In refractive index work, in the observation of melting points, in the study of fatigue failure in metals, in the general examination of alloys, cements, protective coatings, etc., and in the preparation of photomicrographs of certain preparations, this old device now abandoned is really essential, and it is necessary to remove the mirror from the stand and fasten it to a holder of some sort that it may be properly employed.

It is also unfortunate that the objective of small angle, long available working distance and marked penetrating power are not obtainable save at second hand. The modern microscope objective

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is a marvel in its performance, yet it is limited to the study of mounted materials covered wtih a standard cover glass if over a moderate power is required for the examination; but unfortunately, we chemists must work with uncovered preparations and we must sacrifice resolving power for penetrating power and for stereoscopic effects. We need instruments of moderate cost, substantially built, and which will withstand the corrosive atmosphere of most of our industrial laboratories. Thus the third reason for the backwardness of chemists to use the microscope has been the lack of suitable models and accessories. Even our good friends and near chemists, the petrographers, have never gone out of their narrow way, to try to impress the chemist with the fact that the polarizing microscope is an indispensable adjunct of the research laboratory. The modern petrographic microscope is a measuring instrument of great precision. By its means alone a vast number of chemical compounds can be positively identified. The manufacturer of organic compounds, especially, cannot afford to ignore it as a means of increasing the ease of control work. In the hands of a skilled worker this type of instrument offers untold advantages.

The application of microscopic methods to analytical problems should appeal to every chemist. Not only can he perform qualitative chemical analyses easier, but he can measure refractive indices of both solids and liquids, determine melting and boiling points with exceptional accuracy and upon minute amounts of material which cannot be isolated; determine molecular weights and can study the structure of most of our commercial materials. Dr. Harvey W. Wiley, in one of his happy moods, once defined chemistry as "the astronomy of things infinitely small." Our telescope is the microscope. To make suitable progress we must, like the astronomer, construct special instruments for special purposes and like the astronomers we must become specialists in narrow fields within our vast science. We chemists must have analogues to the students of double stars, to the investigators of nebulæ, to the seekers for comets, etc., when this day comes the results reported will be comparable to the discoveries of our astronomical friends. Americans make the finest telescopes in the world. Why not microscopes as well? Unfortunately the scientific world in the United States has been

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obsessed with the idea that no microscopes are worth using, unless made in Europe. We are all to blame for the present difficulties. Can you obtain an ultramicroscope, a luminescense microscope or even a polarization microscope in the United States this 20th day of April? Not one, nor can you obtain condensers, lenses and eyepieces of quartz suitable for photography with ultra violet rays, nor spectroscopic oculars; nor can be purchase a really satisfactory moderate-priced metallograph, although in this line there is hope that instruments will soon be on the market.

If each one of us here tonight will agree hereafter to stand by American manufacturers and buy American made instruments, we will soon have special microscopes and accessories ranking with the best obtainable. Our artisans have no superiors and few equals, but in order that we may persuade them to undertake the construction of the apparatus we require it is essential that we must support them with advice and hard dollars and not with empty applause. It is easy to find fault and refuse to cooperate; but it takes time and tact to call attention to defects and suggest improvements.

If those using special microscopes would stop and consider the care and labor involved in their construction and would be a trifle more tolerant toward mistakes in construction, far greater progress would be made than at present. Let us all agree to try and stimulate the development of American types of microscope which will do our work better and easier, and cease being mere copyists. Let us become "boosters" instead of "knockers".

I have already asserted this evening that the chemical microscope will do more for the chemist than any other instrument or group of instruments, and it behooves me to prove my contention. In the first place microscopic methods are the simplest and shortest for the identification of a compound. Let us assume that the analyst has in his hands a crystalline salt, and by qualitative analysis in the usual manner he decides after about an hour's examination that it contains sodium and phospheric acid—nothing else. It is manifestly a sodium phosphate, but which one? Mono, di or tri? This he can answer satisfactorily by quantitative analysis, and actually only by a determination of Na and PO_4. If, however, he possesses a polarizing microscope, the problem is quite simple. The mono-sodium salt

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is orthorhombic, the di-sodium, mono-clinic, while the tri-sodium phosphate is hexagonal. He can clinch his opinion with one or more simple optical measurements and prove his case by refractive index determinations by the immersion method. Why is it that the chemist never uses refractive index determinations by means of the microscope as an aid in qualitative analysis? It is inconceivable that we have had these methods used for years by mineralogists and petrographers, yet never had sense enough to apply them to our own ends and thereby save ourselves hours of time.

But to go back to our phosphate; had we made the qualitative analysis by microscopic means it would not have taken us an hour, but say not over half that time. We would have been seated comfortably at a table and would have satisfied ourselves in a very few minutes that the salt was di-sodium phosphate badly effloresced and of commercial, not C. P., quality. This case is actually a typical one and very simple. I have selected it because it illustrates quite clearly the way in which a simple salt may be identified. But I hear some of you say this requires a knowledge of crystallography. What of it? If this knowledge will save us time and labor let us by all means do a little reading.

Of all the inorganic salts we will meet with in industrial work only a very few belong to the isometric system and have no effect upon polarized light. Very few tetragonal and triclinic and fewer still hexagonal. There is rarely need for expert training to enable the analyst to properly place the compound under examination in one of these systems. Suppose again the analyst has an inorganic crystalline salt, and under the microscope it separates from water in what appears to be large colorless octahedra which are isotropic. The salt must be an alum, or strontium, barium, or lead nitrate, or one of several chlorostannates. The addition of a tiny drop of nitron sulphate gives no crystalline precipitate, therefore it cannot be Sr, Ba or Pb, or other nitrate. A little calcium acetate gives crystals of calcium sulphate. The salt is presumably, therefore, an alum. A refractive index determination will show which alum, or we can go ahead and test qualitatively for the bases present.

The point I wish to emphasize is that in the identification of many substances a systematic time-consuming analysis is unneces-

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sary. Note well also that all the work is done upon an object slide, that only low powers are employed and the amount of reagent required is negligible. Five grams of practically any reagent used should last an analyst, even in daily examination, almost a lifetime.

I find that the prevailing idea among chemists is that qualitative analysis, by means of the microscope, has for its purpose, the detection of infinitesimal traces of material such that all other methods fail. Although it is true that microscopic methods can be thus employed, by far the greatest points in their favor are the rapidity of obtaining results and the certainty of reaction. Actually the relative proportion of material to solvent is very great, we are apt to be working with high concentrations. We take a fragment of the unknown material, not quite as large as a pin-head, and dissolve it in a minute drop of water or acid. The microscope enlarges this drop of solution until it appears to have the diameter of a twenty-five cent piece. This is almost equivalent to taking a handful of the unknown and dissolving it in a litre of solvent. The identity test is made by adding a reagent which will lead to the formation and separation of a crystalline phase.

It is in the field of organic analysis that the microscope stands without any possible competitor. Differentiation of isomeric compounds, recognition of different degrees of sulphonation, nitration, etc., is so simple in most cases as to be mere child's play. Take the case of the phenol sulphonic acids. Recognition of the different acids, mono or di, ortho, meta or para or mixtures, was a stumbling block for years until Pratt showed that the barium salts were easily differentiated under the microscope. The di-acid salt forms stout mono-clinic prisms, the mono-ortho acid long slender rods, and the para acid tufts of fine needles. Quite recently the microscope was called upon to aid a large plant in controlling the completion of a certain process. It was found that the manganese salt of a certain organic compound crystallized in plates with vivid polarization colors; the other product which it was desirable to eliminate crystallized only with difficulty in sphæro crystals polarizing feebly. A glance under the polarizing microscope showed at once the stage in the transformation of one form into the other. The older method

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of control took not less than twenty-four hours; the new not over twenty minutes.

By far the majority of organic compounds cannot be differentiated and identified without time consuming quantitative determinations. Judicious application of the methods of what Lehmann years ago called crystal analysis yields the necessary information at once.

By means of an electrically-heated stage not only can we determine melting points with greater ease than by the usual methods, but the accuracy of our observations is considerably increased. Amounts of material so small as to be practically invisible to the naked eye can be employed. A further advantage lies in the fact that the melting points of several different substances existing in a mixture may be ascertained without having recourse to long and arduous separations, involving loss of material and time.

Very reliable melting points of fats may also be obtained as well as the boiling and subliming points of small quantities of material.

The advantages of microscopic melting point determinations will become more apparent when we recall that when we separate one compound from another at our laboratory work table, we so proceed that the final products stand upon our table a few inches apart in suitable containers. If we spread a small quantity of the original mixture upon a bit of cover glass and examine the preparation with a magnification of say fifty diameters, a decided space will be seen to exist between most of the different components. Gentle tapping will usually increase this space. To all intents and purposes the magnification has done exactly what we accomplished in our long chemical separation; i. e., removed the components from apparent contact with one another, and interposed space between them. In most instances even very rapid crystallization of two or more salts upon a slide by quick evaporation of their solution will yield a preparation in which the salts will be found to have separated without intermixture, and with a sufficient space between them to allow of a melting point determination being made.

This fact is clearly shown in Fig. 1. Evaporation has been pushed so fast as to force the salts to crystallize in dendritic forms, yet each group of dendrites is clear and distinct. Were such a preparation heated carefully and watched with the microscope, I

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think you will all agree that as one of the components begins to melt it will easily be discerned and will not interfere with the other. When, however, the temperature is raised to the melting point of the second component, the chances are that the two liquids will flow together. Nevertheless the moment of fusion is easily ascertained. If in melting point observations, as I pointed out some years ago, we make use of the polarization microscope, the transition from a solid anisotropic body to a completely fused isotropic body is instantly recognized.

Doubtless that branch of chemical microscopy of greatest general applicability is in qualitative analysis. The addition of a suitable reagent induces the formation of a distinctive compound and its subsequent separation as a solid crystalline phase. These crystals are easily recognized and are so characteristic that there is little danger of mistaking those given by one element or compound for those of another. Add to the distinctive morphology the fact that color also enters into the identification scheme, and it will be even more apparent why microscopic methods offer such ready means of identification. In a large number of cases one and the same reagent will cause distinctive crystals separations with a number of substances. One of the best examples of this is potassium (or sodium) mercuric sulphocyanate, $K_2 Hg(CNS)_4$ (or 2 KCNS. Hg (CNS)2) which gives characteristic crystals with copper, yellowish green (Fig. 2); cobalt, deep blue, (Fig. 3); zinc, white (Fig. 4); cadmium, colorless (Fig. 5); lead, colorless (Fig. 6); manganese, colorless (Fig. 7); gold, yellow; silver tiny colorless; and a red color with iron. Thus the addition of a single reagent will show at once the presence or absence of a number of elements, and at the same time produce an identity test for each, thereby saving an enormous amount of time and material. There are a number of such reagents available, and by carefully choosing them we can complete in a few minutes a qualitative analysis, intricate though it may be.

Time will not permit me to show slides of more than one or more of these multiple test reagents. I have selected cesium chloride, which gives us reactions for bismuth, antimony, tin, copper, silver and lead, and occasionally, aluminum and magnesium. You will note that the crystals obtained are just as different from each other

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and just as easily recognized as those formed by the mercuric sulphocyanates. The slides I now show you are the characteristic reactions for the common acids. You will note that the crystal forms are so different and so easily remembered that there can result no confusion when the tests are properly applied.

The amount of material required for our tests is shown by the tiny fragment clinging to an ordinary No. 7 sewing needle (equivalent to a fragment whose diameter is approximately that of a period (.) on this page). The manner in which a test is performed I have tried to show in this lantern slide, Fig. 8. An ordinary 3″x1″ object slide with the steps in the analysis of an alloy has been photographed natural size in order that the relative sizes of the drops may be better judged. The tiny black spot (0.8 mm. x 0.1 x 0.1 mm.) is a piece of the alloy of the exact size of that employed for the analysis. The large spot at the corner is the space occupied by the solution of the alloy after repeated evaporations with tiny drops of nitric acid to render insoluble any tin present. The nitric acid soluble portion has been decanted to the second spot. The residue has been tested and found to contain tin, antimony, and copper. The nitric acid solution has been divided into three drops as seen on the slide; the first shows the dry residue after finding lead, copper and antimony present; the third spot is what remains on the slide after testing for other elements and finding zinc and iron in traces, in addition to those already found. The fourth spot has been used for testing for the remaining possible metals which were not disclosed in the other tests.

We have thus carried out upon an object slide the entire qualitative analysis of a bearing metal containing tin, antimony, lead, and a small amount of copper, and having traces of iron and zinc present. Actually it took no more time to perform the analysis than it has taken time to tell it. The worker has been seated at a small table and has used less than five cents worth of reagents and gas. Instead of cutting off a small portion of the alloy we could just as well have rubbed it over a piece of ground glass or unglazed porcelain, dissolved off portions of the streak with acid and made our analysis as just outlined. The next lantern slide shows this method together with the results obtained and the time required for the analysis.

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These cases are fair illustrations of what is possible in the saving of time, money and labor, through the employment of microscopic methods.

Other valuable applications of qualitative tests are those involving testing for the purity of precipitates in gravimetric analyses in order to avoid the time and trouble involved in resolution and reprecipitation, in testing for complete precipitation, especially in electrolytic analysis and also in testing for complete washing.

Another valuable application of microscopic chemical methods is in the analysis of the total solid residue in water analyses. We generally speak of the hypothetical combinations present. I do not wish to raise the question of reporting ions or combinations, but I do desire to lay emphasis upon the fact that it is possible and practicable, in most cases, to identify the salts present in the solid residue through their habit and optical properties, providing the work is properly done. I know of several instances where identification of the principal compounds present threw much light upon obscure problems. Traces or more of the heavy metals, such as lead, copper, etc., are far more readily detected by microscopic qualitative analysis methods than by any other means at our disposal.

Water analysts pay altogether too little attention to microscopic examinations of sediments and suspended matters, to the deposits at the bottom of springs, wells and cisterns. Much valuable information is also to be derived from the examination of the muddy ooze at the bottom of streams, ponds and reservoirs, and from the study of the coated sands from rapid filter beds, to learn the extent and character of the adsorption of aluminum hydroxide by the sand grains. When we speak of the "microscopy of drinking water", we generally mean researches upon the flora and fauna giving rise to disagreeable odors and tastes, but this is in reality only a very narrow portion of a huge field which is by no means restricted to biological problems or even to investigations made with the ordinary microscope, since it comprises problems soluble only by means of such specialized instruments as the ultramicroscope and the luminescence microscope.

The remaining lantern slides have been chosen to illustrate the application of microscopic methods to the solution of problems in

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some of the great industries. I can do no more than touch upon them. Permit me, therefore, to merely outline the nature of the information given.

Abrasives: Proper grinding requires adequate speed without undue heating, cutting of uniform depth, wheels which wear well. In other words the selection of the proper sort of wheel and speed for the specific purpose. There enters in addition, the size of particles of abrasive and the nature of the bonding material giving a hard or soft wheel. Much of the manufacturing has been done upon a purely empirical basis and by rule of thumb methods.

Microscopic examinations of particles torn off shows how the wheels have acted. While a similar examination of the ground surface shows the character of the cutting done.

It is surprising how much information may be gained in this way, and how it may be used to guide one in making proper selections.

I will be able to demonstrate that a grinding wheel of a certain kind will tear off the surface of tool steel in such a manner as to heat the steel and draw its temper to such a degree that the particles you will see under the microscope have been fused into tiny spheres. Another wheel rotating with the same surface velocity will cut off the material in ribbons. You will note how few melted fragments are present, as shown by the absence of tiny spherical masses. Such a wheel can be employed for purposes for which the other is obviously unsuited.

Cement, Concrete, Ceramics, etc.—By microscopic examination it is possible to determine the character of the final product, its component parts and their volume per cent; the prevalence of an undue proportion of air and water voids; the thoroughness of wetting of the cement mixtures, etc.

Not less important is the recognition of improper bonding and valuable information is obtainable as to the actual strength of the concrete or its liability to failure. There is here a huge field for the investigator offering untold possibilities.

The whole field of ceramics, both clay products and porcelains, needs intensive microscopic research. Even our ordinary bricks offer a most attractive subject for the investigator.

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The thickness of glaze and the thoroughness of its bond with the body-making material can readily be determined. I have prepared slides of two high-grade porcelains; in one of these, Fig. 9, you will note the glaze is thick and between it and the porcelain are a vast number of tiny gas voids. In some cases these gas voids penetrate the glaze as infinitely fine capillary tubes. The other porcelain of far higher quality has a much thinner glaze and as you see, (Fig. 10), has almost no gas voids, the bonding is almost perfect. Examinations of this sort, employed in the industry, go a long ways to improve the products turned out.

Foods and Beverages: Doubtless the earliest application of microscopic methods by the chemist was in the examination of foods, food accessories and drugs for adulteration or deterioration. Examinations of this sort are based largely upon vegetable and animal histology, and differ but little, if any, from the ordinary technique of histology. No other methods are available to accomplish the ends in view, and we can therefore assume that in this line at least the microscope is indispensable, training in the necessary technique imperative, and every food analyst and expert must perforce train himself to undertake studies of this sort.

This field of activity is so well understood and so firmly established that we can dismiss it without further comment. But there is in this whole question of the microscopy of foods and drugs, another phase, which chemists have greatly neglected, that of the amazing possibilities of microscopic qualitative and quantitative analysis. To even enumerate the list of substances whose detection becomes simpler, more certain and much more rapid than by our ordinary routine tests, would require more than the remaining time at my disposal. These "micro" tests are applicable to organic, as well as inorganic substances, as I have already pointed out. We have nothing better than microscopic methods for the detection of the poisonous metals, for the recognition of the organic acids, for the detection of preservatives, for the vegetable alkaloids, glucosides, and other active principles of plants; nor can we find other methods available for the quantitative analysis of starch mixtures and for similar analyses of mixed powders and meals of vegetable origin.

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The recognition of our commercial synthetic drugs also is considerably simplified. In fact it is no exaggeration to say that proper analyses of this sort cannot be conducted unless the microscope is employed.

In the canning industry, especially that employing tin cans and other containers, the microscope gives information of untold value. Soldered and crimped joints yield up their secrets, as also the tinned surface or other protective coating which may have been applied to metal or paper surfaces. The possibilities, are in fact, without end.

Metallurgical Industries: The microscopic study of metals and alloys has been so firmly established within the last few years, and the close relation between structure and physical properties so generally recognized and its importance proved in practice, that I need not spend time upon this question. Although these methods have been placed upon a firm foundation in the iron and steel industries, there is much work to be done in the great field of commercial alloys. We generally think of heat treatments in terms of steel only. As a matter of fact many alloys may be greatly improved by carefully conducted heat treatments. In order that such work may be properly done, microscopic studies of structure are imperative. This is well shown in photographs of a copper, zinc, and of a copper-aluminum alloy, which I will show you.

Often the microscopic appearance of a roughly polished and etched specimen taken in conjunction with a hasty qualitative analysis will give the investigator all the information he may require to deduce the quantitative composition, and to make a shrewd guess at the physical properties.

A most fruitful field leading to improved practices is the study of welds and brazes under the microscope. At least one expert in welding by means of oxy-acetylene owes much of his remarkable success to microscopic studies of welded materials.

Not infrequently the microscope shows that a poor product is due to improper temperatures of casting or coating, and not to bad raw materials or wrong percentage composition. This is especially the case in babbitts and in tinned goods. We have here photographs

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showing the great difference in the structure of a babbitt cast at too high a temperature and the same one poured just right. It is obvious that in the one case friction will be considerably greater than in the other, particularly if in a high speed bearing.

Paints, Pigments, Protective Coatings: The microscopic studies of materials falling in this class may be classed under three heads: (1) The examination of the raw materials, (2) that of the coated surfaces and (3) studies of the methods and results of applying the coatings to the objects to be protected.

Briefly stated the raw materials under the microscope (chiefly pigments, etc.,) reveal their source and nature; often the process of manufacture and their suitability for the purposes for which they have been purchased. Take for example the mere question of size in the selection of the pigments for a mixed color paint. It is a simple matter to obtain a whole series of different shades by using, say two pigments and having them vary in the ultimate size of their particles, although the per cent by weight of each remains constant. The actual shape of the particles also probably seriously effects the length of time the paint really acts as an efficient protector, especially in paints containing silica, graphite or both. The microscope also throws light upon the nature of the changes taking place in the pigments of paints on being exposed to air, light and the weather. A good illustration is to be found in the study of the cause of the darkening of lithopones.

Examination of the weathered, coated, objects, both of the surfaces and of section, cut through the thin films of coatings, not infrequently will permit the formation of an immediate opinion as to the quality of the paints or coatings, and the skill of the workmen who applied them.

Too little attention has been paid in the past to the study of sections. I believe you will all be interested in the preparations I have to show you. I have selected them because they exhibit in a striking manner the differences between good and bad paints, and between good and bad workmanship in their application. You will also note that the appearance under the microscope of these samples of japanned steel show very clearly the superiority of the

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one method of baking over the other: in actual practice the superior surface costs less to apply than the inferior and wears longer and better.

The microscope in the hands of the chemist dealing with this class of commercial material enables him to rapidly evaluate the products handled by his firm, to improve faulty products and to determine whether the materials supplied to the trade are being properly applied.

Nor must we omit from mention the valuable information we may obtain from the microscopic study of woods to which fire or decay-proofing substances have been applied.

I have thus far failed to mention one great industry based largely upon microscopic control—the Paper Industry. Practically, an analysis of paper without reference to its ultimate composition as shown by the microscope is of little or no value. Actually much paper is manufactured and employed for various purposes without studies under this instrument. As a necessary consequence we frequently meet with paper-fibre goods manufactured with little regard for the ends to be attained other than to sell at a profit. A fair criticism of our American paper products are that they are too good. That is to say, the quality is higher than need be, and the cost to the consumer is greater than it should be for many of the objects to be attained. By that I mean that a less expensive product would serve equally well and not infrequently better. This is poor business and poorer conservation. Let me cite a case in point.

A few years ago a firm manufacturing a product (which must be nameless, since the investigation was conducted in confidence) appealed to the laboratory for advice. Their product, and that of their competitors also, was failing to stand up unoer new conditions of use. In desperation the chemist of the firm wanted to know whether the microscope would reveal the source of the trouble. Critical study for a day or two of new and failed material showed that changes made by the paper firms were probably the cause of the trouble. A commercially better grade of paper was being supplied. The matter was taken up with the paper firm. The answer was quick and to the point. The paper supplied was the highest grade

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that could be produced at that price and further they didn't propose to have any men, mere analysts, tell them their business. They had been manufacturing papers before the questioning men were born, etc., etc. In fact the same old story and the same old trouble with many well-meaning American firms. All you industrial men have had similar experiences. I need not go further.

A small firm was prevailed upon to make a paper of a kind which, it was believed, would eliminate certain features which the microscope seemed to indicate to be the cause, or at least one of the causes of the troubles. This new paper was then treated in the proper manner and tested out. The results were so satisfactory that a contract was placed to take the entire output of the paper firm with specifications as to the kind of paper needed. The net result was that a product was obtained in which, not only were the old defects eliminated, but the cost of production was decreased, the final profit greatly increased, and the stability of this industry assured. But I am not sure that the paper firm which lost a large contract is even today convinced that the new methods of microscopic investigations are of value.

There is little doubt that similar conditions obtain in many of the other varied paper-fibre industries. Microscopic methods are the only ones which enable the analyst to identify the nature of the paper and to indicate its fitness and adaptability for the specific uses to which it will be put.

The technique for the recognition of the nature of the fibres present and for their quantitative determinations are fairly well established and are on the whole, quite satisfactory. But a phase of microscopic investigation has been neglected. A study of the finished surface with reference to the uses to which the paper is to be applied. A study of paper surfaces with vertical illuminator and with oblique light yields most interesting results. Were these methods more often employed there would be, in many cases, a decided modification in certain papers on the market.

The Textile Industries: Like paper, the analyses of textiles and the recognition of the fibres of commerce are dependent entirely upon the proper application of microscopic methods. At the present

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time no other satisfactory methods are available for differentiating between the species of fibres employed, the specific treatment they have received, or the loom arrangement by which the yarns have been woven into fabrics. In not a few instances the information thus obtainable may go even farther and disclose the nature of the method used in dyeing the yarn or the fabric. Uneven adsorption, variable penetration, etc., are easily recognized. The skilled investigator may go even farther and discriminate between different qualities of the same species.

The technical microscopy of the textile fibres is still in its infancy, its literature teems with inaccuracies and contradictions. Too little attention has been devoted to the investigation of the reactions of reagents and the selection of proper differentiating stains, and the potential possibilities of dark field condensers having very oblique ray illumination (ultramicroscope) and of luminescence illumination (ultra violet rays) have not yet received the attention they deserve.

By way of illustration of what the microscope reveals, I call your attention to several lantern slides selected to show how neglect to employ this instrument led to the failure of a manufacturer to reproduce a fabric which his firm was called upon to manufacture because of war conditions. The reproduction involved producing a similar yarn from like fibres, a similar weave in the fabric, and a similar colored printed pattern. You will see that in no case was he successful and that his different attempts were a waste of time, material and energy, since he apparently lacked the fundamental microscopic information necessary for success.

I trust that in these rambling remarks I may have converted some skeptics to a belief in the importance of chemical microscopy in our industries, and may stimulate a wider interest in a branch of chemical analysis whose value has been greatly underestimated and whose development has been sadly neglected.

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EXPLANATION OF PLATES

PLATE XI. Fig. 1. Crystallization upon an object slide even so rapid as to lead to the formation of dendrite crystals rarely results in contact between the crystal groups. Melting points may often therefore be determined without the necessity of separating constituents. In the field photographed are crystals aggregates belonging to the isometric and to the monoclinic systems.

PLATES XI, XII, XIII, XIV. Figs. 2, 3, 4, 5, 6, 7. A single reagent often gives very different crystals with different elements. The illustrations show the crystals produced by the action of potassium mercuric sulphocyante upon solutions of salts of the elements listed below. Thus, by adding a single reagent the presence or absence of many substances may be at once indicated effecting a saving of time, labor and material.

Fig. 2. Reaction with Salts of Copper, giving $Cu(CNS)_2 \cdot Hg(CNS)_2 \cdot H_2O$, color, greenish yellow.

Fig. 3. With salts of Cobalt, $Co(CNS)_2 \cdot Hg(CNS)_2$; color, deep blue.

Fig. 4. With salts of Zinc, $Zn(CNS)_2 \cdot Hg(CNS)_2$; color, white.

Fig. 5. With salts of Cadmium, $Cd(CNS)_2 \cdot Hg(CNS)_2$; colorless.

Fig. 6. With salts of Lead, $Pb(CNS)_2 \cdot Hg(CNS)_2$; colorless.

Fig. 7. With Manganous salts, $Mn(CNS)_2 \cdot Hg(CNS)_2$; colorless.

PLATE XIV. Fig. 8. Illustrates the amount of space required to perform the complete analysis of an ordinary commercial alloy (Babbitt). The photograph is that of the end of a standard 3x1 in. object slide upon which an actual analysis has been effected. The spots show where the tiny fragment of alloy was dissolved, reagents applied, and decantation of drops from one place to another practiced. Since the quantities of material required are small the time consumed and the amount of reagents required are reduced to a minimum.

PLATE XV. Figs. 9, 10. Show the application of microscopic methods to the study of ceramic glazes. Fig. 9 shows a thick glaze improperly bonded and filled with gas bubbles. Fig. 10 a thin glaze closely adherent to the porcelain and containing very few gas bubbles.

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Fig. 1

Fig. 2

Plate XI

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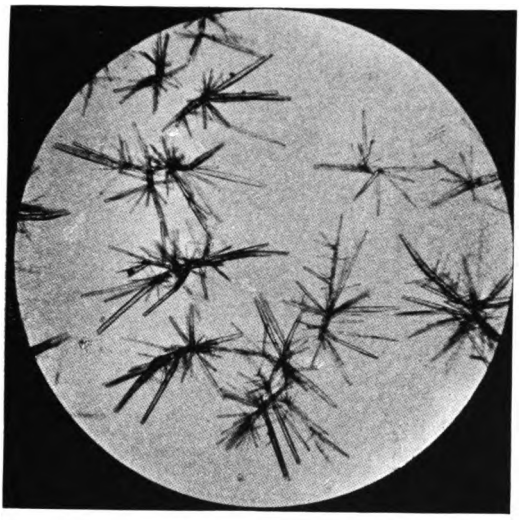

Fig. 3

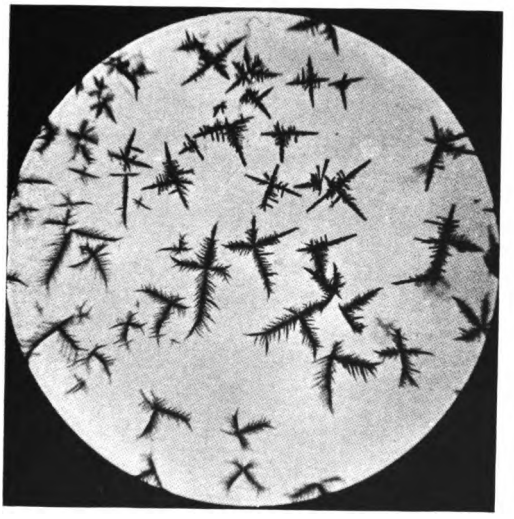

Fig. 4

Plate XII

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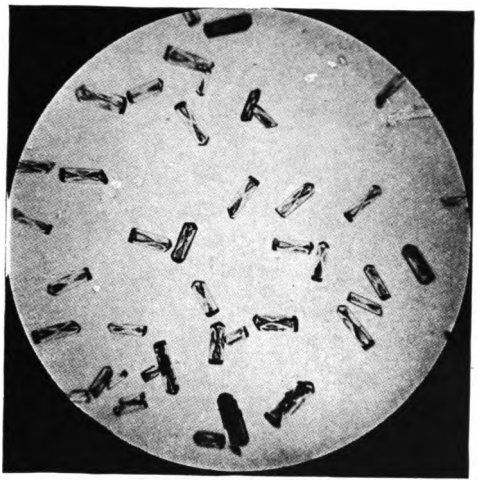

Fig. 5

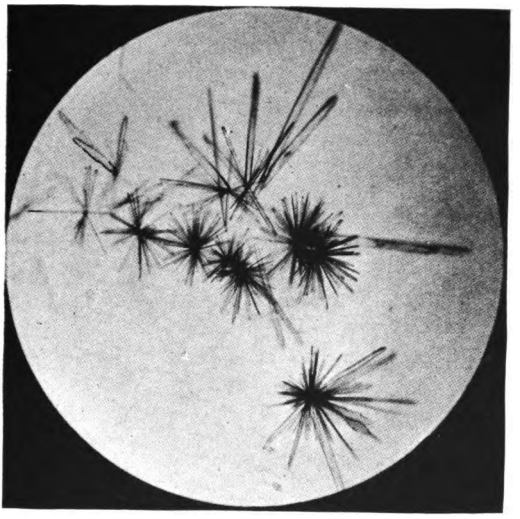

Fig. 6

Plate XIII

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Fig. 7

Fig. 8

Plate XIV

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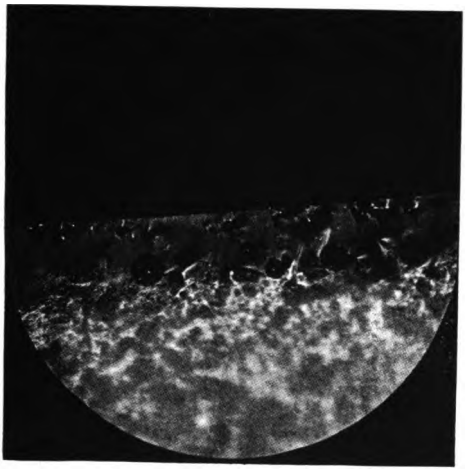

Fig. 9

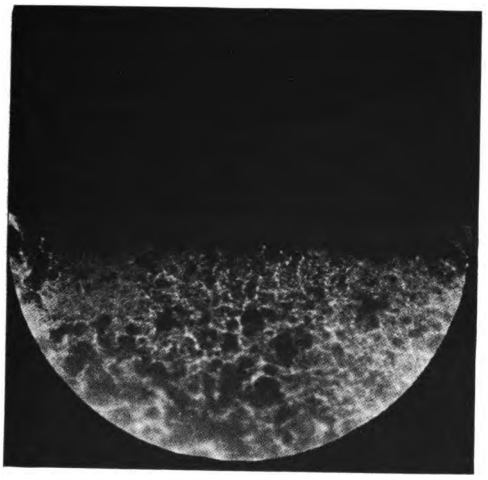

Fig. 10

Plate XV

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SOME COMPARISONS BETWEEN NUCLEI OF NERVE CELLS

By William A. Hilton

Nuclei were compared from a large number of animals, chiefly invertebrates. In this short paper nuclei alone are drawn. No special method has been applied at this time. In all cases strong fixing reagents and strong stains were used, such as Flemming's fluid or mercuric cloride. The stain used was hematoxylin in most cases. Slight differences which one or another reagent gives will not be entered into at present. The figures were made from preparations which seemed to be typical cells for the animal. In the methods used no great differences were seen in the appearances of the nuclei except that by some methods they stained more intensely.

The study is particularly directed towards a comparison of the distribution of the basichromatin in the nuclei. All drawings are at the same scale and drawn with a camera lucida with the best oil immersion objective available.

Many of us appreciate the advances which Biology has made through the aid of chemical interpretations and physical and chemical explanations. Some are inclined to belittle pure morphological studies, but it seems in many cases that we have to come back to the purely anatomical and morphological investigations in order to advance. Great attention has been given to the dividing period of cell activities, but are not the periods between division more important in many respects? Is it not probable that this is the time when the nature of the cell is determined? Evidence has gradually been accumulating to show that the nucleus and the materials within it are closely related to the cell body and its activities.

The nucleus is probably not concerned with conduction or the special activities of the neurone, although it may have to do with nutrition. Even in Protozoa Verworn '88 (1), denies the nucleus being the seat of any psychic activity as expressed by motion. The nucleus seems to have nothing to do directly with irritability; frag-

(1) Zeit. f. wiss. Zool. llvi.

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ments without nuclei respond as readily to stimuli as other parts. That the nucleus controls or conditions the character of the cell body seems true to some degree, but just how much, opinions differ. In those cells which retain their power to divide the chromatin content remains large, in those which differentiate, this chromatin falls proportionately, but in some cells it may be restored once more when certain stimuli or special nutrition prepares them for division again. Nerve cells and some others do not as a rule divide after their mature life is reached, and in these the chromatin content is especially reduced. E. Rohde '11 (2) calls attention to the reduction of chromatin in other than germ cells and compares the loss through polar body formation to the loss in nerve cells, yolk cells and in the extreme condition in the red blood cells of mammals.

Although nerve cells do not divide they may give out growths during this period as shown by G. Marinesco and J. Minera '14 (3), who studied a culture of spinal ganglion cells *in vitro*. The connective tissue cells did grow by multiplication, but the nerve cells did not divide although they gave out processes. From other evidence it seems possible also that in the adult central nervous system there may be growth or change of position of fibers to make new connections.

M. Muhlmann '11 (4), has studied the structure of nerve cells at different ages in mammals. He finds at first a large amount of chromatin in the nucleus, later it became less, some goes to form a sort of nucleolus. The tigroid substance of the cell body gradually increases. Later Muhlmann '14 (5) studied the composition of Nissl's granules and found that they have a nuclein content along with globulin, but the nuclein of Nissl's granules differs from that of nuclei because the tigroid substance is soluable in alkalis and the chromatin of the nucleus is not. Just what relation the lost chromatin of nerve cells bears to the cytoplasm remains to be clearly determined, but that there is a relation seems clear from the work of many.

(2) Zeit. f. wiss. Zool. xcviii.

(3) Anat. anz. 1914.

(4) Arch. mic. Anat. lxxvii.

(5) Arch. mic. Anat. llvi.

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H. Stauffacher '10 (6) studied the nuclear structure in animal and plant cells and found the oxychromatic matrix substance of the nucleolus connected by internal nuclear bridges with the oxychromatin of the nucleus and that, with the oxychromatin of the cytoplasm. Basichromatin always has an oxychromatin foundation. Nucleoli arise from oxychromatin and pass to the nucleus and from there into the cell substance. Nuclein has to do with growth and metabolism. He does not believe in a nuclear membrane. The drawings and photographs of Stauffacher look very convincing. I have found similar conditions in some germ cells. J. Schaxel '10 (7) speaks of the regulative emmission of chromatin to the cell from the nucleus. W. Knoll '10 (8) has studied the leucytes in bone marrow. Nuclear bridges connect contractile parts of the nucleus with contractile parts of the cytoplasm. There are many other papers with similar evidence which suggest either a close chemical interrelationship or definite organic connection between cytoplasm and nucleus.

That nuclei may receive again from the cytoplasm, or that the cell body aids the nucleus in the growth of its chromatin seems clear from the recent work of V. Danchakoff '16 (9). "Accumulations of basophilic substance are found in the cytoplasm * * *. The small achromatic nucleus of the mature egg both after fertilization and at the beginning of parthenogenesis seem to exert a strong attraction on the basophilic accumulations found in the cytoplasm. This substance is soon displaced and localized around the nucleus. At the same time a radiation around the nucleus is perceptible, which is apparently the expression of the flowing of the basophilic substance towards the nucleus * * *. The close relation between the true chromatin bodies within the nucleus and the basophilic chromatic substance in the cytoplasm is easily traced."

In a study of a large number of nerve nuclei such as shown in figs. 1 to 17 and fig. 22, it is found that in most nuclei there is but a scanty distribution of nuclear material which is proportionately

(6) Zeit. f. wiss. Zool. xcv.

(7) Verh. viii Intnat. Zool. Kong. zu Graz. 1910.

(8) Zeit. wiss. Zool. xcv.

(9) Jour. morph. vol. xxvii, no. 3.

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less in the larger nuclei of cells that have very large cell bodies. In most cells there is a well-marked, usually clear nucleolus. In many there are many fine strands of linin visible, usually radiating from the center of the nucleus. The smallest animals have the smallest cells and these have the smallest nuclei. Very often the smallest nuclei do not look much like those of nerve cells, such as those of Podura (fig. 13) and Aphorura (fig. 12), seem more like neuroblasts or young cells although their chromatin content is small. In the larger animals there was considerable variation noted in cells from the same animal, such as in L. grandis, fig. 6, Trantula fig. 10, Corydilis, fig. 16.

In all the preparations probably the more granular nuclei are those from neuroblasts and a few of the clear nuclei may be of neurogolia cells. In the nerve nuclei of a young salamander Desmognathus, fig. 22, the chromatin is very abundant and the cells with such nuclei are capable of active multiplication. In figs. 23 and 24 from muscle and connective tissue cells, the scanty chromatin would be insufficient for immediate division. Figs. 18 to 21 are from another salamander, a larval Amblystoma. Fig. 18 from the retina might soon have more chromatin and divide, one of the epithelial cell nuclei of fig. 19 could soon divide and fig. 20 from the blood has a mass of chromatin and the muscle nucleus of fig. 21, is not near division.

No more than a general idea of the distribution of the chromatin in the nuclei of nerve cells can be learned from these figures. A general type of nucleus shows fairly well in all but the smallest and youngest specimens. The type is that usually recognized for the nerve cells of vertebrates, that is a large rather clear nucleus with a nucleolus which is also rather clear. There seems to be nothing especially peculiar to nerve cell nuclei which would prevent the cells of the adult from receiving a stimulus of some sort to increase the chromatin of the nucleus and allow further division of the cells.

Whether the arrangement of the chromatin means anything or not in determining the nature of the cells cannot be told from these preparations, but I do not doubt that this as well as any particular

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or peculiar arrangement of the nuclear parts may be of importance at one time or another in determining the nature of cytoplasmic manifestations.

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Claremont, California.

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EXPLANATION OF FIGURES

All figures drawn by the aid of a camera lucida by means of an oil immersion objective. All enlarged eight hundred diameters.

Fig. 1. Nerve nuclei from the starfish, Orthasterias.
Fig. 2. Nerve nuclei from the starfish, Piaster capitatus.
Fig. 3. Nerve nuclei from the crustacean, Nebalia.
Fig. 4. Nerve nuclei from the crustacean isopod, Lygida.
Fig. 5. Nerve nuclei from the crustacean isopod, Lygida 4mm. long young.
Fig. 6. Nerve nuclei from the crustacean decapod, Loxorhynchus grandis.
Fig. 7. Nerve nuclei from the pycnogonid, Lecythorhynchus marginatus.
Fig. 8. Nerve nuclei from the whip-scorpion, Tythreus pentapeltis.
Fig. 9. Nerve nuclei from the pseudoscorpion, Chelifer.
Fig. 10. Nerve nuclei from the tarantula spider.
Fig. 11. Nerve nuclei from the thysanuran insect, Campodea.
Fig. 12. Nerve nuclei from the collembolan insect, Aphorura.
Fig. 13. Nerve nuclei from the collembolan insect, Podura.
Fig. 14. Nerve nuclei from the collembolan insect, Sminthurus.
Fig. 15. Nerve nuclei from the thysanuran insect, Machilis.
Fig. 16. Nerve nuclei from the larval insect, Corydalis.
Fig. 17. Nerve nuclei from the ascidian, Tunica.
Fig. 18. Nucleus from one of the retinal cells of the larval salamander Amblystoma 15 mm. long.
Fig. 19. Nuclei from epithelial cells of larval Amblystoma, 15 mm. long.
Fig. 20. Nucleus from the blood of a 15 mm. larval Amblystoma.
Fig. 21. Nucleus from the muscle of a 15 mm. larval Amblystoma.
Fig. 22. Nuclei from brain of a young salamander, Desmognathus.
Fig. 23. Nucleus from striated muscle from young Desmognathus.
Fig. 24. Nucleus from connective tissue from young Desmognathus.

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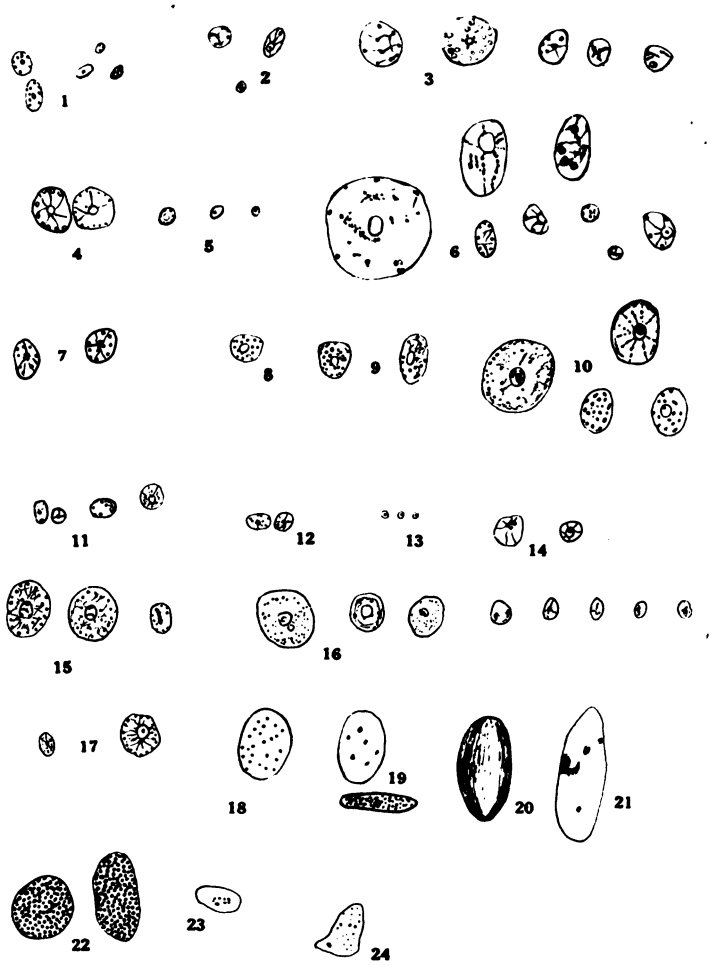

PLATE XVI

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

NOTES ON THE CULTURING OF MICROSCOPIC ORGANISMS FOR THE ZOÖLOGICAL LABORATORY

General.—The culture of micro-organisms, especially the Protozoa, is an old topic among biologists. Leeuwenhoek in his quaint language records not only the finding of Protozoa in various natural waters but also in infusions of pepper. He was thus the first to make cultures of the Protozoa. Leeuwenhoek's cultures were largely due to a series of accidents or to haphazard experiments. Many zoölogists of today use this haphazard method of making cultures. It is the usual practice to collect a mass of vegetable material from a pond, stream or other body of water. This mass is placed in almost any kind of a dish, and left in strong light or in darkness, covered or uncovered as long as the preparator can stand the odor. From these haphazard methods he gets some surprising results but quite frequently not at the time when wanted. If he has made these cultures with some particular protozoan in mind, he is quite likely to find the species mixed (if he finds it at all) with several other species. When through using these cultures or when they are spent he throws them out and makes more in the same haphazard fashion.

These have been and are yet to a large extent the methods of culturing Protozoa as practiced by the zoölogist. Bacteriologists, however, have developed very careful methods of culturing bacteria and some few species of Protozoa. They make use of media containing known and measured ingredients, kept under controlled conditions. The response of the organisms to particular media and to particular conditions is characteristic and the habit of growth

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is an aid in diagnosis of the species under observation. The bacteriologist cannot use haphazard methods and secure results. Botanists also are making use of exact methods in the culturing of plant organisms as algæ, fungi and even higher green plants. The use of exact methods by botanists has been promoted by the fact that many of them are trained in bacteriological methods and in the exact methods of plant physiology. Their research in the life histories and in the metabolism of lower plants has demanded the use of exact methods. The zoölogist, too, must come to the use of quantitative methods in the culturing of many of the lower forms of laboratory animals. He is, however, more or less dependent on the bacteriologist and the botanist to show him the methods of rearing the food organisms for his Protozoa or his worms. The zoölogist can, indeed, help himself very greatly if he will learn to use the cultural methods of the bacteriologist and the botanist. It is not enough that some zoölogists make use of these methods but having worked out methods that are successful they should by early publication make their methods available to others. The writer has no sympathy with the disinclination of some workers to make their methods known. There is a real need among amateur zoölogists, teachers and investigators for a greater knowledge of technical methods and such information ought to be made readily available.

Some criticisms of the old methods may be given and some suggestions offered concerning food supply, dishes, light, inoculation and culture media.

Food Supply.—One of the great faults of the old methods of culturing micro-organisms was in the failure to maintain a stock of food materials in cultures that had begun to yield. When the culture was made it was heaped high with food materials which were never replaced as they became exhausted by the action of bacteria. With depletion of the organic matter the crop of bacteria or of unicellular algæ which furnished pasturage for many of the Protozoa rapidly diminished in numbers and the Protozoa decreased to the vanishing point. For this reason it has been the custom to bring in cultures every few days in the hopes of having one culture ready when wanted. It should be borne in mind that if the number of animals in a culture is to be maintained their food supply must be replen-

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ished. This can be done by adding hay, manure from various sources or manure solution, or other vegetable or organic material which does not quickly become acid in the process of decay. Rapid souring of culture medium is fatal to most Protozoa. The writer has never been very successful in growing Protozoa in media containing much of such carbohydrates as occur in wheat.

Culture Dishes.—Even the kind of a dish in which the culture is maintained is of importance. The best dishes are shallow and have a diameter considerably greater than their depth. Covered glass dishes ca 200 mm. in diameter by 80 mm. in depth and known as bacteria dishes or culture dishes are excellent but not indispensable. The writer has used Stender dishes 50 mm. in diameter by 30 mm. in depth with success although a small culture is more difficult to maintain than a larger one. Fruit jars, battery jars and even aquarium jars are satisfactory if the liquid of the culture is not too deep. Earthenware jars and crocks are satisfactory for forms preferring the dark. Keep all cultures covered.

Light.—As to light, diffuse light or even darkness may be used for most forms except those which feed on a green or blue-green plant or which like Euglena need light for photosynthesis. Even in these cases the diffuse light and more even temperature of a north window are to be preferred to direct sunlight and the accompanying violent fluctuations of temperature.

Inoculation.—If in the wild cultures the desired organisms appear in numbers new cultures may be inoculated with a small quantity of material from the old culture, taking precaution to add the food organism also and to avoid contamination with other organisms. Thus one may become reasonably free from the necessity of frequent trips to pond or stream to collect vegetation for cultures. The culturist should study the food habits of the organism and strive in preparing the new culture to provide the proper medium for the development of the food organism and then he must provide proper conditions of light, temperature and gaseous exchange. If by observation or experiment he can determine the ingredients to be used in his culture and the conditions for the growth of the food organisms he should be able to maintain cultures almost indefinitely.

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Culture Media.—Various culture media have been suggested for general and special Protozoa cultures. The writer has confined his efforts to a few media. One of the most successful of these for general cultures is made of sterile hay and filtered tap-water. To prepare this medium timothy hay is made into small compact bundles which are tightly wrapped in several layers of cheese cloth. The bundles are sterilized once in a steam autoclave at 15 to 17 lbs. pressure for 15 minutes or more, then dried over a steam radiator or in a drying oven. This sterilization is not intended to kill bacteria or spores of fungi but to kill encysted Protozoa if any be present. The tap-water is filtered through a paper filter, preferably into sterile dishes. The idea here is to free the water of any Protozoa which are always present in tap-water of this city but not necessarily to get rid of the bacteria. Ten grams of the sterile hay to two liters of filtered tap-water makes a very satisfactory medium for many kinds of Protozoa. These proportions, however, can be varied considerably. When a culture has been started in this medium by inoculation from a previous culture it may be maintained by the addition of one or two grams of sterile hay per week and as much filtered water as is necessary to maintain the original level of liquid.

Boiled hay solution has been tried repeatedly and has never given good results, apparently because of the development of toxic substances. It usually soon acquires an odor somewhat resembling butyric acid and when in this condition if Paramecia are placed in it they die in a very few minutes.

Manure solution made by boiling fresh horse or cow manure or human feces in tap-water and diluting with filtered tap-water has given excellent results in culturing Euglena and some unicellular green algæ as Scenedesmus. Any of these may serve as food organisms for some of the Protozoa. Unfortunately the proportions in which the manure solutions were made have not been kept. It has, however, been the experience of the writer that the proportions may be widely varied. Manure solutions may be added to the hay cultures with beneficial results.

The writer has found the filtered tap-water superior to rain-water which is lacking in many of the salts necessary for most organisms. Distilled water has never been used because it has been

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simpler to use the filtered tap-water than to add salts in the proper proportions to the distilled water. Boiled tap-water is inferior to filtered tap-water probably because boiling precipitates a large proportion of the salts and drives off the oxygen and carbon dioxide. In the event that the tap-water has been treated with chlorine it may be necessary to use filtered pond or stream water or spring water.

Culture of Amœba.—Two of the writer's students, Miss Knisely and Mr. Welch, have worked out methods of culturing ameba on solid media. The results of this work were published in in these Transactions, vol. 34, pp. 21-25.

The writer's work in culturing ameba has been incidental to the securing of materials for class use and some of the results attained have been more or less accidental. Ameba cultures have been secured in many ways of which only a few are related.

During the summer of 1916 small amebæ, probably soil amebæ, occurred in great abundance in a culture made from unwashed radishes pulled from a damp garden. No efforts were made to maintain the culture and the amebæ soon disappeared.

On Aug. 29, 1916, some lily-pads and pond water were taken from First Sister Lake, near Ann Arbor. This culture yielded among other Protozoa a fair number of large amebæ. On Dec. 29, 1916, a sterile-hay-filtered-tap-water culture was made up in a bacteria dish and some scum from the preceding culture was added. In this culture a slender diatom and the ameba established themselves and have thriven to date. This ameba is a large rounded sluggish form measuring nearly 100 microns in extreme dimension. Its surface is wrinkled. It puts forth few pseudopodia, has a thick hyaline ectosarc, finely granular entosarc, large contractile vacuole, and a well-defined ellipsoidal nucleus within a wider clear area. Specimens have usually been very numerous in this culture and since there have been very few other organisms present it has been excellent for student use. To this culture were added at intervals of one to several weeks a few grams of sterile hay until June 21, 1917. At this time the culture was in good condition. The diatoms were very numerous on the bottom of the dish where the sluggish amebæ were abundant. There were also many more active amebæ

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resembling *A. proteus.* From June 21 until Aug. 30 no hay was added to this culture and it was not examined meanwhile. On the latter date amebæ of the above mentioned kinds were found but not abundantly. About 2 grams of hay were added Aug. 30 and on Sept. 19 both kinds of amebæ were quite abundant.

On Jan. 9, 1917, a subculture in a sterile-hay-tap-water medium was made from the culture above mentioned. For a long time it yielded amebæ altho less abundantly than the parent colony, but on June 21 it was producing quantities of diatoms upon which large numbers of the sluggish ameba were feeding. This culture had more hay than the parent culture. It was uncared for from June 21 until Aug. 30 when a few amebæ were found. Hay was added Aug. 30 and on Sept. 19 the amebæ were more abundant.

The above cultures have always been kept near a north window in diffuse light and have always been kept covered with a glass plate. Recent comparison of these amebæ with amebæ secured on Sept. 12, 1917, from the original source of these cultures shows that they have not changed in form or size.

Culture of Euglena.—Euglena occurs most abundantly in foul waters and is well known as an inhabitant of sewage-polluted waters. Euglena cultures have been maintained in luxuriance with almost no attention on the sterile-hay-filtered-tap-water medium for more than a year in the diffuse light of a north window. They also have been transplanted into manure solutions where they have produced a dense green scum over the surface of the water and down the sides of the jar and as far above the water on the sides of the jar as the film of moisture extended. After a time large numbers of these Euglenæ become encysted. It has been found by Dr. A. F. Shull of our laboratory that such Euglenæ soon emerge from the cyst if placed in a fresh manure solution. Thus the providing of Euglena for class use which formerly was the bugbear of the general zoölogy course is now a very simple matter.

Culture of Tetrastemma.—A fresh water nemertean worm which resembles Tetrastemma and has provisionally been assigned to that genus has appeared in cultures taken from First Sister Lake, near Ann Arbor, on July 12, 1916. Until September, 1916, this culture was maintained in a covered pint fruit jar, then was put in

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a bacteria dish with filtered tap-water and some sterile hay. Tetrastemma was first noted in November. The culture was maintained by adding a few grams of sterile hay at intervals of one or more weeks. During most of the winter this organism was abundant. On March 23, 1917, this worm was present in large numbers, but by June 21 its numbers were greatly reduced. On Aug. 30 a single specimen was found in a subculture.

Culture of Pristina.—In the culture in which Tetrastemma appeared and at the same time the oligochæte worm, *Pristina sp.* appeared and was maintained in considerable abundance until late February and although on March 23 it was on the decline it was still present in cultures on June 21. Subcultures were made from time to time during the winter in which this worm thrived. The species has disappeared in these cultures during the summer. The cultures of Tetrastemma and of Pristina were maintained just as Paramecium cultures were maintained. In fact during the greater part of the time Paramecia were very numerous in all the cultures of these worms.

University of Michigan,
Ann Arbor, Mich.

GEORGE R. LARUE.

EFFECTS OF THYROID ON PARAMECIUM

Shumway (J. Exp. Zool., Apr., 1917) cites a number of interesting results from culturing Paramecium in emulsions and suspensions of thyroid. Among these are,—that the division rate is increased 65% over the controls; that this acceleration of rate is greatest at the time when the controls themselves are dividing most rapidly, and hence the curves show the same general rhythms as those of the controls; that the number of contractile vacuoles increases from two to three, indicating a disturbance of the excretory function; that large vocuolation takes place in the protoplasm, which is believed to have an excretory significance, altho the vacuoles do not pulsate. They were often observed to burst, freeing their contents internally.

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Of all the hormone-secreting glands the thyroid is the only one to produce the above results. Boiling does not destroy the activity of the material. Iodine and iodothyrin do not produce it.

When the treatment is discontinued the strain of Paramecium reverts to the division rate of the controls. The other mutations brought about by the feeding do not seem to persist.

ARE CONJUGATION AND ENCYSTMENT NECESSARY?

Mast (J. Exp. Zool., July, 1917) concludes that neither conjugation nor encystment is necessary for the continued existence of Didinium. By starting new groups of lines from old ones both near and remote from encystment and propagating these in parallel series, it seems that these data have no constant or appreciable effect on fission rate, or on variations in this rate. In these experiments a group was carried an average of 1646 generations without encystment. In one group of lines, 721 generations after conjugation and 196 generations after encystment, a mutation suddenly appeared in which the rate of division for 315 days was more rapid than the slower lines in the ratio of 836 generations to 634.

EXTRA CONTRACTILE VACUOLES IN PARAMECIUM

Hance (J. Exp. Zool., July, 1917) gives the account of the origin of a race of Paramecium with supernumerary vacuoles, apparently arising thru subjection to temperatures higher than usual. The race was unusually strong, large, and resistant to extreme conditions. The extra vacuoles, ranging as high as five, usually occurred in the posterior end of the cell. They may increase during the vegetative life of the individual; and the number is modified by the rate of division, the age of the culture medium, and the presence of katabolic products in the environment. The author holds that the "potentiality for this organ has not been lost, tho the extra vacuoles may not actually appear for several generations."

PRESERVING FISH WITHOUT ICE

Sherman's Fish Sterilizing Co. owns a process for preserving fish without ice (Pacific Fisherman, July, 1917) which is claimed

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to keep the fish fresh and firm with all the original flavor for a fortnight or longer. The process takes about 3 hours. The fish are first put in a cooling tank filled with water and brought to a low temperature, where they are left for one-half hour. It is next placed in a tank of cold sea water or fresh water strengthened with salt. In order to prevent freezing in the brine the solution is kept agitated by pumping. It is claimed that the low temperature salt solution closes up the surface pores, preventing saturation and also acting as an antiseptic protection on the outside.

It is claimed that the process is cheap in installation and operation and will enable the small producer to get his catch on the market in good condition, thus aiding increased production and safe marketing.

CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION IN ROTIFERS

Hickernell (Biol. Bul. June, 1917) gives a most interesting account of the desiccation changes in the rotifer *Philodina roseola,* with special reference to cell behavior. Attention is called to the fact that rotifers are resistant not merely to drying. They are resistent to great cold (even — 40° F does not kill them), to great heat, to strong brine, or to rapid changes from one to another of these unfavorable conditions. Murray has summarized this hardiness thus: "Such is the vitality of these little animals [certain south polar Bdelloids] that they can endure being taken from ice at a minus temperature, thawed, dried, and subjected to a temperature not very far short of the boiling point, all within a few hours."

The structural changes in Philodina during desiccation are summarized as follows: The animal as a whole contracts into a form similar to that of extreme contraction in life; no special protecting membrane is formed,—contrary to the common view; the tissues and cells maintain their identity during the drying process; the cytoplasm becomes more dense, and the chromatic part of the nucleus changes from a single large karyosome, separated from a distinct nuclear membrane by a clear space, to chromatic network at the periphery with the clear space internal.

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The author believes that some metabolism goes on during the dry condition. The evidence for this lies in the fact that food granules in intestinal cells disappear during desiccation in a manner similar to that seen in starving specimens.

It is suggested that the movement of the chromatin to the periphery of the nucleus during desiccation facilitates cell oxidations during the period. Their change in position takes place at the very beginning of the drying process. On recovery from desiccation the changes are the reverse of those described above.

The acceleration of reproductive activity often noted in rotifers soon after recovering from drying is credited to an increase in ovarian nuclei that occurs during the period of recovery. When death results from desiccation any of the following causes may operate:—mechanical injury due to too rapid drying; starvation due from a lack of reserve food material; poisonous effects of metabolic products; insufficient time during early drying to allow the nucleo-cytoplasmic reorganization.

THE FUNDUS OCULI OF BIRDS

In a most attractive atlas Dr. Wood has presented the results of his studies upon representatives of all the leading orders of birds, including more than 100 species. He rightly takes the view that a group of animals with such highly developed and varied vision as birds offers a most fruitful field for the study of all the elements entering into the structure and activity of the eyes.

His method provides for study with the self-luminous ophthalmoscope, macroscopic examination of excised eyes, and microscopic examination of special portions. Owing to the fact that the mydriatics usually used for man have little or no direct effect on the sphincter iris musculature of the bird, it was found necessary to secure dilation by the use of such agents as galvanism, nicotine, curare, and of drugs that render the bird unconscious without actually killing it. It was also found that maximum dilation is to be had a few moments before and after the death of birds, where these are being killed for detailed studies of eye-structure.

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The evidences are abundantly manifest of close and effective cooperation between the author and the artist, Mr. Arthur Head of London, in the remarkable series of colored plates of some 58 species of birds and 3 species of reptiles, not to mention more than 100 black and white drawings.

The chief variable features of the fundus oculi capable of study by their combination of methods are:—the color and form of the internal background; the shape, size, and degree of development of the pecten; the optic nerve entrance; the choroidal and retinal blood vessels; the details of distribution of the retinal elements, including the opaque nerve fibres; the shape and size of the optic discs or areas of acute vision.

Some of the outstanding conclusions of the author are:—

1. Such examination of the fundus as has been indicated furnish very different and characteristic fundus pictures for different groups of birds; sufficiently so in many cases for the immediate recognition of the species. These fundus pictures will rank with other taxonomic indicia in classification. The author is able to suggest certain broad classifications of fundi.

2. There is great variety among birds in respect to the size, shape, and position of the areas of distinct vision. This localization corresponds strikingly with the habitats and habits of the birds,—especially to manner of getting food, escape from enemies, migration, reproduction, etc.

3. Domestication or prolonged captivity seems to bring changes in the eye of birds. These abnormalities include choroidal diseases, opaqueness of media, less constancy in many of the variable elements of the fundus.

4. Study of the fundus of birds in comparison with those of other vertebrates may be expected to throw light upon the origin and kinship of the group.

The chapters are:—1. Introduction; 2. Summary of Conclusions; 3. Collection, Selection, and Preparation of Material; 4. Review of Anatomy and Physiology of the Fundus Organs; 5. Ophthalmoscopy; 6. Ophthalmoscopy of the Fundus in Living Birds; 7. Macroscopic Appearances; 8. Effects of Domestication on the

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Fundus Oculi; 9. Appearance of the Fundus Oculi in Various Orders of Birds; 10. Classification of Ocular Fundi; 11. Ocular Fundus in Relation to Classification of Aves; 12. Relation of Reptilian to Avian Fundi; 13. Atlas of Colored Plates of Fundus Oculi in Birds.

The mechanical and artistic qualities of the book are of the first rank. It is an ungracious thing to refer to small matters of incompleteness where the larger things are so painstakingly and admirably cared for. There is, however, incompleteness and lack of uniformity in the use of subdivision headings and numerals in Chapter VI particularly, and a lack of correspondence between these and the table of contents.

The Fundus Oculi of Birds, Especially as Viewed by the Ophthalmoscope, by Casey Albert Wood. Illustrated by 145 text drawings, and 61 colored plates. The Lakeside Press, Chicago, 1917. Price $15.

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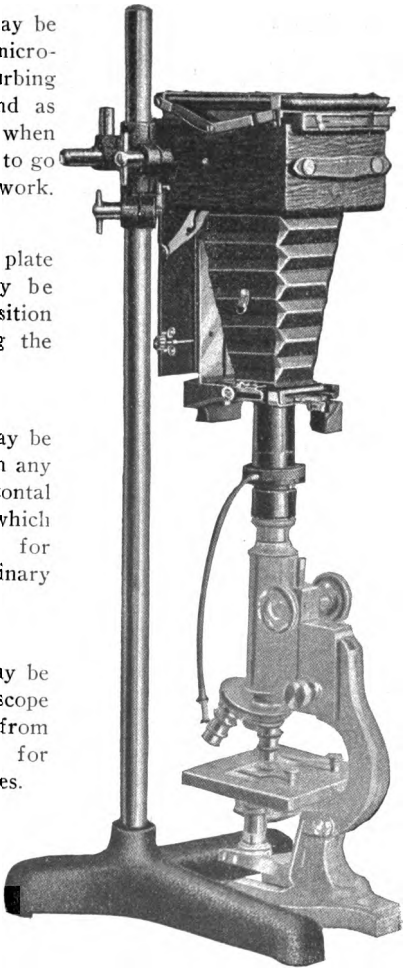

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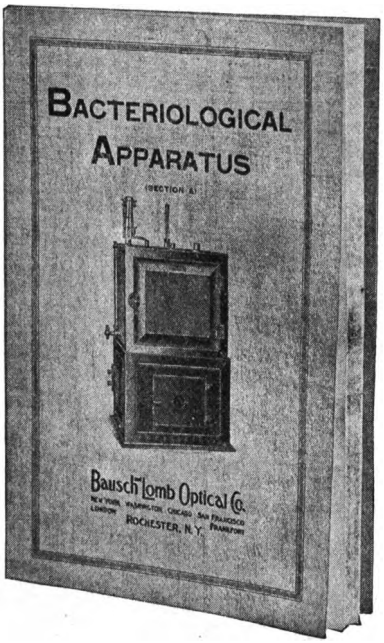

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